عنوان المشروع باللغة العربية	التيلومير والحمض النووي ردا الضرر TRF2 من sheltrin دور مكونات التيلومير في تطور سرطان القولون والمستقيم في المرضى السعوديين ATM
عنوان المشروع باللغة الإنجليزية	The Role of telomere sheltrin components of telomere TRF2 and DNA damage response ATM in colorectal cancer progression in Saudi patients
المشرف الرئيس	Abeer AlDbass
التخصص الدقيق للمشرف الرئيس	proteins and Molecular Biology
المشرف المساعد	Sooad Al-Daihan
المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا	12 months
Abstract or synopsis of the proposal (200 words or less):	One of the most common cancer in Saudi Arabia is colorectal cancer in which the incidence has been shown to be increasing gradually over the past few years. Among the various changes occur in can cer cells is telomere dysfunction which consider as an important early event associated with genetic instability. The function of telomeres include the stabilizing the ends of chromosomes, protect them from end-to-end fusion and mediate chromosome pairing during cell division. Human telomeres are composed of TTAGGG tandem repeats in addition to groups of proteins called shelterin complex, which protects chromosome ends, regulates telomere length, recombination, and DNA damage checkpoints. Shelterin is composed of TRF1, TRF2, POT1, TPP1, TIN2, and RAP1. The loss of telomere protection is the root cause of the premature aging The shelterin subunit TRF2 plays a key role in suppressing the telomere-associated DDR through its binding to and inhibition of ATM kinase 6 and 7. ATM is the apical kinase that in response to few DSBs activates many subpathways of the DDR. Although it has been proposed that shelterin inhibits the DDR at telomeres and not elsewhere in the genome, this attractive proposal remains elusive because ATM is not detectable at telomeres.
Hypothesis or scientific justification of the proposal	Several reports have indicated that the altered expression of TRF2 proteins is associated with tumor progression in various human carcinomas, including lung, stomach, adrenal and pancreatic cancer; the altered expression has also been identified in malignant hematopoietic cells and colorectal pre-neoplastic lesions. It has been found that TRF2 upregulated in human cancer cells. Consistent with a potent oncogenic role of a high level of TRF2, its downregulation in a variety of cancer cells reduces tumorigenicity, whereas overexpression of TRF2 favors oncogenesis. Although a high level of TRF2 expression in cancer cells is expected to maintain a sufficient level of telomere functionality to prevent DDR activation and chromosome instability and to sustain cell proliferation and stem cell function, very little is known about its exact role in colorectal cancer oncogenesis. The aim of this study is to investigate the role of TRF2 and how it will affect ATM. The expressional level of (TRF2, and ATM and chk2) and

	protein level determination using immunohistochemistry in colorectal cancer in Saudi patients
Specific objectives	1. Determining the expressional level of (TRF2, ATM and Chk2) in colorectal cancer and its matched control 2. Protein level determination using Immunohistochemistry of TRF2, ATM and Chk2 3. Methylation status of TRF2 promoter using Methylation Specific-PCR
Methodology & Major Techniques to be used	Patient samples: Colorectal tissues from Saudi patients will be obtained from the collaborators and clinicians as per the guidelines of IRB. Patients attending the oncology department at KKUH will be examined by the oncologist and routine examination performed. Patient group would comprise men and women with colorectal cancer of all ages and stages of the disease (n=20). Surgical core biopsy and adjacent normal specimen will be obtained prior to treatment and immediately stored in RNAlater solution (Ambion) for DNA and RNA extraction. Nucleic acid Isolation: High–molecular-weight DNA/RNA will be obtained from freshly collected colorectal cancer samples, matched normal samples and blood samples (DNA) using Qiagen DNA/RNA mini prep kit and Qiagen nucleic acid extraction kits according to the manufacturer's protocol. Quantitative RT–PCR: Expression will be assessed by quantitative RT–PCR in duplicate using SYPR green chemistry (Applied Biosystems) and specific primers TRF2, ATM and chk2. The relative amount of RNA will be calculated with the CT method. Gene expression will be normalized with the GAPDH , and the level of expression of the tumor sample will be compared with the mean level of the gene expression in normal liver tissues and expressed as an n-fold ratio. MSP-PCR: The promoter methylation status of TRF2 of some samples will be determined using EpiTect Bisulfite Conversion Kit from Qiagene and then amplification using PCR. Immunohistochemistry: IHC for TRF2 and ATM will be performed in representative colon tumor and normal tissue. Briefly, deparaffinized and rehydrated sections will be subjected to microwave treatment in 10mM sodium citrate buffer, pH 6.0, for antigen retrieval. The sections will be incubated overnight at 4° C in a humidified chamber with the primary antibody TRF2 and ATM. The detection will be performed with a labelled streptavidin–biotin immunoperoxidase detection system and the immunohistochemical staining will be developed with 3,30-diaminobenzidine substrate. Omission of the primary antibody incubation will be used as negative control.
Availability of Samples	YES

If the answer is no, kindly justify	
Availability of Chemicals	YES
If the answer is no, kindly justify	
Availability of Instruments	YES
Availability of Ethical Approval (if needed)	YES
Recent References	• Mounir El Maï, Kay-Dietrich Wagner, Jean-François Michiels, Damien Ambrosetti, Arnaud Borderie, Sandrine Destree,……, Eric Gilson1 (2014).The Telomeric Protein TRF2 Regulates Angiogenesis by Binding and Activating the PDGFRβ Promoter.Cell Press 1047–1060 • Buscemi, G., Zannini, L., Fontanella, E., Lecis, D., Lisanti, S., & Delia, D. (2009). The Shelterin Protein TRF2 Inhibits Chk2 Activity at Telomeres in the Absence of DNA Damage. Current Biology, 874-879. • Weber, A., & Ryan, A. (2014). ATM and ATR as therapeutic targets in cancer. Pharmacology & Therapeutics, 124-138.